

Please cancel claims 33-35 and 40 and amend claims 32, 36-39 and 41-44.

In the claims

1-17 (cancelled)

18. (withdrawn) A nucleic acid comprising a DNA sequence that substantially corresponds to SEQ ID NO:1 wherein at least one mutation has been introduced in the sequence corresponding to the β -bridge.

19. (withdrawn) The nucleic acid according to claim 18, wherein the mutation occurs at a site which encodes the PA in the β -bridge.

20. (withdrawn) The nucleic acid according to claim 19, wherein the mutation at the site that encodes PA is a substitution or deletion such that the substituted nucleic acid encodes a single amino acid, a dipeptide, a tripeptide and a tetrapeptide.

21. (withdrawn) A nucleic acid according to claim 17, wherein the at least one mutation results in an amino acid change selected from PA/A, PA/AA, PA/PGA, PA/PAPA, Δ PA, PA/K, PA/G, PA/D and PA/P.

22. (withdrawn) A vector encoding the nucleic acid of any of claim 18 through 21.

23. (withdrawn) A host cell containing a vector of claim 22.

24. (cancelled)

25. (withdrawn) A method for modifying enzyme catalytic activity, comprising:

(a) selecting an enzyme having two catalytic centers connected by a β -bridge, the catalytic centers being located at reciprocal stereo-geometric positions in the enzyme;

(b) changing the reciprocal stereo-geometric position of the two catalytic centers by introducing a mutation into the β -bridge; and

(c) modifying the catalytic activity of the enzyme.

26-28 (cancelled)

29. (withdrawn) A method of forming a shotgun cloning library, comprising

(a) incubating a modified DNA cleaving enzyme according to claim 1 with a DNA to form non-sequence specific cleavage fragments of the DNA that are ligatable; the ligatable DNA being capable of insertion into a vector for cloning in a host cell; and

(b) forming the shotgun cloning library.

30. (withdrawn) A method for mapping nicks in a duplex DNA, comprising;

(a) incubating a modified DNA cleaving enzyme according to claim 1 with the duplex DNA in a manganese-containing buffer;

(b) permitting nicking to occur across from a pre-existing nick site to form fragments of the duplex DNA with single strand overhangs; and

(c) mapping the nicks in the DNA.

31. (cancelled)

32. (currently amended) A composition, comprising:

~~a recombinant protein having DNA cleavage activity, the protein further characterized by two catalytic centers separated by a β -bridge and having at least 35% amino acid sequence identity with SEQ ID NO:12 for a T7 Endo I, wherein the recombinant protein when compared with T7 Endo I has:~~

~~(a) — reduced toxicity for an *E. coli* host cell; and~~

~~(b) — an amino acid sequence in the beta bridge that differs by at least one amino acid from SEQ ID NO:12.~~

a variant T7 Endo I polypeptide having endonuclease activity, and comprising an amino acid sequence that differs from the amino acid sequence of its parent T7 Endo I polypeptide solely by one or more mutations in a β -bridge corresponding to amino acids 44-49 of SEQ ID NO: 12.

33-35 (cancelled)

36. (currently amended) The composition according to claim 32, having an altered activity, the altered activity further comprising: (e) ~~greater DNA cleavage activity of the recombinant protein variant T7 Endo I~~ in a manganese-containing buffer compared with DNA cleavage activity of parent T7 Endo I in the manganese-containing buffer.

37. (currently amended) The composition according to claim 36, wherein ~~one or more~~ the DNA cleavage activities ~~are~~ is selected from the group consisting of: cleavage of a cruciform DNA; nicking opposite a preexisting nick site; nicking next to a mismatch in the DNA resulting in double strand cleavage; and non-specific nuclease activity.

38. (currently amended) The composition according to claim 32, having an altered activity, the altered activity further comprising: (e) reduced DNA cleavage activity of the ~~recombinant protein variant~~ T7 Endo I in a magnesium-containing buffer compared with DNA cleavage activity of parent T7 Endo I in the magnesium-containing buffer.

39. (currently amended) The composition according to claim 38, wherein ~~one or more~~ the DNA cleavage activities ~~are~~ is selected from the group consisting of: nicking opposite a preexisting nick site; nicking next to a mismatch in the DNA resulting in double strand cleavage; and non-specific nuclease activity.

40. (cancelled)

41. (currently amended) The composition according to claim 32, wherein the ~~difference of at least one amino acid in the β -bridge~~ one or more mutations is a substitution or a deletion at a site corresponding to a Pro-Ala (PA) dipeptide in the β -bridge corresponding to amino acids 44-49 of SEQ ID NO:12.

42. (currently amended) The composition according to claim 41, wherein the ~~substitution~~ one or more mutations is a single amino acid substitution, a dipeptide substitution, a tripeptide substitution or a tetrapeptide substitution at the PA; or wherein the deletion is a deletion of PA dipeptide, or wherein the deletion is a deletion of, Pro(P) ~~and~~ or Ala(A).

43. (currently amended) The composition according to claim 42, wherein the PA dipeptide is substituted with an Ala (A), a Lys (K), a Gly (G), an Asp (D) or a Pro (P), an Ala-Ala (AA) dipeptide, an Ala-Gly-Ala (AGA) tripeptide, or a Pro-Ala-Pro-Ala (PAPA) peptide, ~~or a deletion of PA.~~

44. (currently amended) A kit containing at least one of: the composition of claim 41; an isolated nucleic acid comprising the composition of claim 32 \pm ; a vector comprising the nucleic acid; or a host cell comprising the vector.

45. (previously presented) A method of determining whether a DNA substrate has a single nucleotide polymorphism (SNP), comprising:
 (a) contacting the DNA substrate with the composition according to claim 32; and
 (b) determining from the cleavage product whether the DNA substrate has the SNP.

46. (previously presented) The method according to claim 45, further comprising: identifying which nucleotide forms the SNP.

47. (previously presented) The method according to claim 45, further comprising: identifying the location of the SNP.